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Ultrasound Imaging and Ovarian Follicles Growth of Mares at Nirwana Equestrian Club

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Abstract. *Mares in the estrus cycle by the form of reproductive determine the precision of ovulating time so that efficient to the appropriate time of artificial insemination for breeding. The determination requires supporting medical equipment such as Ultrasonography during the ovulation. This research was conducted to know the ovarian follicle imaging of mares during estrus with Ultrasonography that helps through presume ovulation and the appropriate time to proceed with artificial insemination. This observation was conducted at Nirwana Equestrian Club Semarang, from October until December 2023. The sampling is collected by purposive sampling method where the sample is specifically the mares while in their estrus symptom. The collected data and information are processed descriptively using laboratory equipment such as Ultrasonography. The observation collected the mares physical estrus symptoms such as urination, vulvar pulsination, and vaginal fluid discharge. Significant development was observed from the initial follicle size of 5.34 cm to 5.61 cm in one of the samples with the examination interval within 18 hours before the occurrence of the corpus luteum. The results were proved by 9 samples of mares that had been measured with ultrasound during their estrus, and undergoing pregnancy by one attempt of artificial insemination. The optimum time for artificial insemination for mare is during ovulation where the effective precision is supported by ultrasonography.*

Keywords: *Artificial insemination, Estrus, Follicle, Ultrasonography*

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INTRODUCTION

The low conception rate due to the difficulty of detecting symptoms of estrus, increases the risk of various inaccuracies (Santoso, 2014). Action is needed to decrease the risk of reproductive failure by medical support using appropriate equipment to reach efficiency. This attempt also supports the accuracy of natural mating or artificial insemination. The examination and detection is using ultrasonography to know the follicular estrus cycle which will support efficiency in reproductive actions. The use of ultrasonography will visualize the mare's follicular ovaries condition, thereby it can measure the appropriate time for horse mating. The imaging of mare's estrus will result in an appropriate action decision that will result in the pregnancy of mares in one mating attempt.

RESEARCH METHODS

Design of Research

This research was carried out by observing estimates using laboratory equipment.

Sampling Methods

The determination of sampling using *purposive sampling* where samples are taken based on certain characteristics by the research objectives.

Procedures

1. Observation of Mare in Heat

The observation of mare in heat can be by paying attention to the mare's reproductive organs in general, which could be winking, secreting mucous, lifting its tail, being aggressive, or even not giving any signs at all. This research was determined through observations from one estrus period to the next period. The whole number of horses at Nirwana Equestrian is 42 and there are 27 mares, including 20 mares that are sexually mature and not pregnant so that the physical symptoms of heat can be observed.

2. Ultrasonography Examination

Examination of ultrasound starts with rectovaginal, by inserting hand and linear probe into the rectum and cervix.

3. Ultrasonography Result Record

Measurement of the examination results will be centimeters (cm) of the follicle diameter, measured from each dimension of the farthest follicle's surface distance. A comparison is made for each measurement where the dimensions of the follicle will change and increase over time. The measurements are carried out until the follicle is

known to be a corpus luteum which the luteal phase starts and determine the appropriate time to do a reproduction examination to gain mare pregnancies.

4. Reproductive Examination

Mares at Nirwana stable that are intended for breeding will undergo artificial insemination or natural mating with selected stud, while sporting mares that are in heat will not be bred and the period will be allowed to pass. Artificial insemination can be performed when it's the time of ovulating which is 24 hours before the estrus ends thereby mares that have 5 5-day estrus period long can be bred on the fourth or fifth day of the estrus period (Kusmayanti, 2013).

Procedures

This study data result will be processed descriptively. Follicle imaging data within screenshot documentation of the ultrasound device and the size of the follicle diameter in centimeters (cm).

RESULTS AND DISCUSSION

RESULTS

1. Physical Characteristics of Estrus Mares

The way the estrus behaves in heat is different for each individual, but in general, the symptoms of the estrus that can be observed are winking or pulsing the vulva, releasing mucous, acting aggressively, urinating large amounts, and not giving any estrus symptoms at all, which is known as silent heat. Estrus can be directly observed through the physical symptoms of the mare, and this study was carried out on approximately 20 sexually mature and non-pregnant mares.

Tabel 1. Estrus Symptoms

Estrus Symptoms	Quantity (mare)	Precentage
Release Mucous	11	55%
Aggressive	4	20%
Silent Heat	2	10%
Urinating	3	15%
Total	20	100%

2. Ultrasound Imaging

Ultrasound procedures are carried out to monitor the development and the increase of follicle diameter at examination intervals every 6–10 hours. This study was taken from the last three intervals before the corpus luteum occurs within 4 to 5 days of the estrus period.

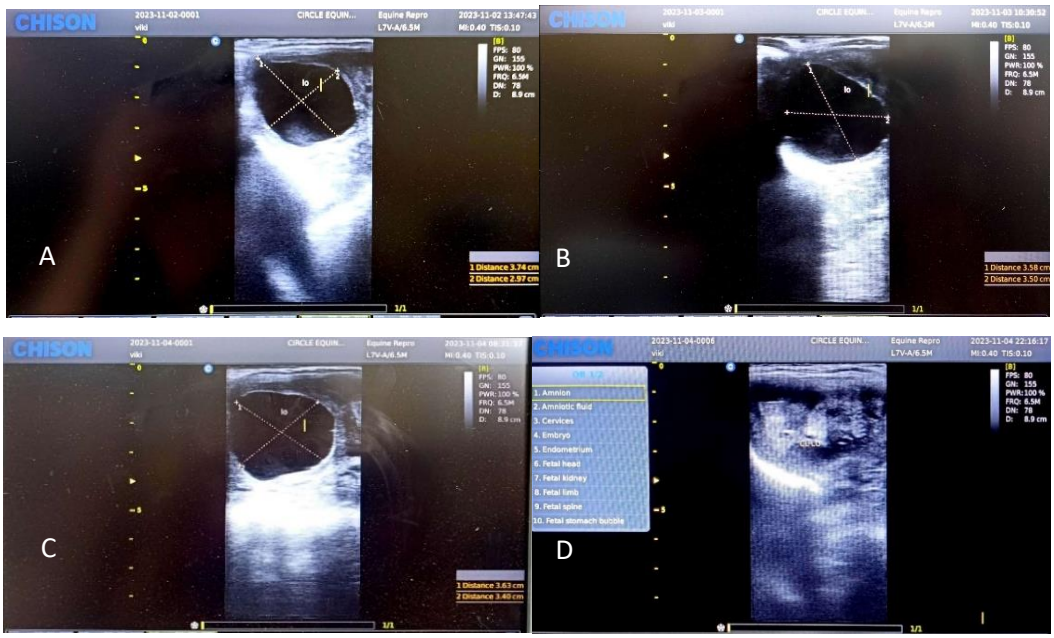

Figure 1. (A) the first examine follicle average 3,35 cm (B) next interval avregae follicle 3,54 cm (C) last examine before corpus luteum reach 3,52 cm (D) lysis, corpus luteum.

3. Follicle Development Examination Results

The table of follicle development taken is the average diameter of the (right and left) selected dominant follicles measured by the ultrasounography feature in centimeter.

Tabel 2. Results

Examine Intervals	Sample Follicle Diameter (cm)								
	H1	H2	H3	H4	H5	H6	H7	H8	H9
I1	3,16	5,34	4,06	4,55	5,32	4,41	4,65	4,31	4,07
I2	3,54	5,45	4,24	4,66	4,03	4,54	4,58	4,25	4,29
I3	3,51	5,61	5,65	5,1	4,17	4,63	5,15	4,33	3,9
Desc.	CL AI	CL AI	CL AI	CL AI	CL AI	CL AI	CL AI	CL AI	CL AI

Description :
CL (corpus luteum)
AI (artifical insemination)
I (intervals)
H (horse)

DISCUSSION

1. Physical Characteristics of Estrus Mares

Estrus symptoms in mares are different for each individual due to various factors such as race, physiology, and the climate in which the individuals live. Although there are differences in variations of estrus signs between each individual, they tend to be the same (Yulianto, 2016). There are 42 horses at Nirwana stable and 27 mares of them. This research uses mares samples that are sexually mature and not pregnant, so there are 20 potential estrus mares. The symptoms displayed by heat mare at Nirwana stable are from Kusumayanti's research in 2014, which found that mares in heat release mucous, urinate, and wink the clitoris. One of the mare of this research sample was that it did not resist emitting mucous and did not wink when it was brought close to the stud. The differences between each individual also determine the differences in their follicular development. This happens because the follicles produce estrogen, which causes symptoms of ongoing estrus (Morton, 2023).

In the case of proving the symptoms of heat in this study, all mares at Nirwana stable experienced the same physical symptoms of heat. In Indonesia's general type of heat can be categorized as 3A (*abang, abuh, anget),* which means redness, edema, and warmth. The first symptom that can be felt while examining the edema in the vulva area is physical throbbing. This occurs due to the estrogenic stimulation from the reproductive hormone (estrogen) which produces edema in the tendons and tissues of the uterus (Ismudiono, 2010). The warmth generated by the vagina is also caused by secondary sexual characteristics that increase vaginal temperature and pH (Wijayanti, 2020).

2. Ultrasonography Procedure: Prepare

The ultrasound used is the Chinson ECO 2 with a 15-cm-long linear probe, which can be used rectally to get imaging of the ultrasound result (Santoso, 2014). The materials and tools needed for an ultrasound procedure are red palpation gloves, paraffin, or cooking oil to facilitate the entry route through the rectum during the ultrasound process, a clamp collage, and a halter or horse harness. The first step in carrying out an ultrasound procedure is to put the mare in a clamp cage and then clean the rectal tract of remaining feces, as shown in Figure 2. In carrying out the palpation process, the hands involved must be sterile and have removed all jewelry or other objects attached to the hands. The use of red palpation gloves is for disguising blood stains if there is any blood during

palpation. The use of palpation gloves is completed with the use of paraffin or cooking oil to facilitate entry through the rectum. Removal of remaining feces in the rectum is carried out slowly and manually.

Observations are by inserting a probe into the rectum and directing it at the rectal wall, where it meets the urinary bladder, to obtain an image of the uterine cornua. Once the uterine junction is discovered, the probe will be directed to the right or left to get an image of the ovary with a dominant follicle or a larger follicle. Follicles that have a greater chance of being effective in the artificial insemination attempt will be focused on during the whole procedure.

Figure 2. Palpation to remove the remaining feces in rectal canal

3. Ultrasound Imaging

Ultrasound observations include measuring the diameter of the follicle and corpus luteum (CL). The difference between black and gray can be seen in ultrasound images, namely isoechogenic (gray) and hypoechogenic (black). An increase in follicle diameter in mares in heat is followed by a routine examination at 6-12 hour intervals during the estrus period which begins on the second day after the first appearance of the estrus signs. At the end of the estrus period, the follicles will change in diameter, the maximum follicles become deformed until they turn into CL. Regular examinations at every interval observe the phenomenon of ovulation with the rupture of the Graaf follicles in the ovaries (Talakua, 2020). The dominant follicle that ovulates can also cause a decrease in follicle diameter because the follicle begins to lyse and release hormones within it. Measurements are made by drawing a line perpendicular to the edge of the follicle using the available dist feature to obtain a number displayed on the screen.

The average of the measurements of each follicle edge will be an estimate of the size of the follicle diameter at one examination interval. The status of the follicle as it develops into CL will be an isoechogenic (grey) image that contains follicular fluid

consisting of hormones including FSH, LH, prolactin, oxytocin, vasopressin, estrogen, and progesterone, and several enzymes and proteins to be the most appropriate time to carry out artificial insemination and natural mating (Gillian, 1991). The CL phase which produces progesterone will prepare the uterus to keep the incoming egg so that the lysis period is the main indicator for observing the effectiveness of pregnancy when it is known that the follicle has undergone lysis through ultrasonography.

4. Follicle Development Examination Results

Changes in the estrus behavior and the diameter of the follicle to the corpus luteum were observed every day until ovulation occurred and the data taken was the last interval before the corpus luteum occurred, it is once every 6-10 hours on days 3 to 4 after the first symptoms of estrus appeared. The data taken is the last three examination intervals before the corpus luteum occurred. Based on the results of observations that the representative example of the sample, H1 in Table 2, found significant follicle development, it is experiencing an increase in the size of the first recorded diameter of 3.6 cm, this figure was obtained from the average perpendicular diameter between the edges of the follicles. In the second examination interval, it is 6 hours after the first examination, it was found that the follicle had grown to 3.54 cm, so the difference in diameter between measurement intervals was 0.6 cm. The diameter of the follicles increases by 3 to 5 mm every day (10 mm=1 cm). This characteristic also relates to the texture of the follicles which will become more flexible every day due to the ovulation (Mottershead, 2023). At the third examination (18-hour interval) the follicles in sample H1 had reached a maximum of 3.51 cm. In one of the samples H9, it showed a continued decrease in the development of follicle diameter to 3.9 cm from the previous interval which was at 4.29 cm. The decrease in size causes lysis due to the reduced volume of follicular fluid so that the follicles cannot be measured because the image is abstract (isoechogenic) indicating that ovulation has occurred (Mottershead, 2023).

In the breeding practice at Nirwana Stable, horses that are usually in heat for five days will then be mated on the fourth and fifth days or until there are no longer any physical symptoms of heat by the female. The absence of physical signs of estrus by the mare may signal the end of the estrus period which concludes with ovulation. This method is believed to increase the effectiveness of pregnancy when horses are bred. Based on the research, observing the CL phenomenon is a benchmark when artificial insemination is

the most effective way for breeding. This is because all samples that were carried out by artificial insemination using fresh semen or frozen semen were positive for pregnancy. Pregnancy examination also uses ultrasound 15 days after the day of artificial insemination and there is an embryo sac as in Figure 3. Within 15 days, ultrasonography can capture an image of the embryo sac that has formed, a sign of successful artificial insemination that occurred due to suspected ovulation. Pregnant mares do not go into heat in the next estrus cycle, but this does not reduce the possibility that pregnant mares will still go into heat in the first month or two of pregnancy.

Figure 3. Embryo Sac within 15 days of artificial Insemination

CONCLUSIONS

Ultrasonography of the mare's ovarian follicle development is appropriate at the time of ovulation, which is approximately within the last 18 hours before the end of the estrus cycle, which is marked by the appearance of the corpus luteum on ultrasound. There is a mare in one of the samples is in heat that proven by significant follicle development, starting with a follicle measuring 5.34 cm in the first examination interval and ending with a size of 5.61 cm in the final interval before the corpus luteum occurs. The use of ultrasound equipment is explained and carried out at the discretion of the veterinarian.

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